CHAPTER 1

Introduction

1.1 Significance of the study

Solubility improvement of a drug is recognized as one of the most challenging tasks in pharmaceutical area. Great effort has been paid on it not only on the drug discovery but also throughout the formulation development and the production process. The significance of the solubility evokes from the strong influence of the solubility on bioavailability. The Biopharmaceutics Classification System (BCS) introduced by drug regulatory agencies classifies drug substances into four classes based on their aqueous solubility and their ability to permeate the biological membrane (Lobenberg and Amidon, 2000). According to this classification system, drugs belonging to Class II and Class IV emerge poor aqueous solubility. For these drugs, their absorption is restricted by the slow rate and low extent of dissolution. A poor soluble drug is defined by a drug solubility of less than 100 microgram/ml. It is equivalent to that of practically insoluble according to USP terminology. Any of newly discovered drug substances have solubilities of this range. To achieve the goal of maximum bioavailability, more effective solubilization techniques should be progressively investigated.

A number of conventional solubilization techniques have been applied for improving the solubility of drugs. These techniques includes cosolvency using mixed solvents, pH adjustment; salt formation (Fini et al., 1995), crystallinity reduction or

polymorphism alteration (Handcock and Zografi, 1997), micellar solubilization (Miyasaki et al., 1981), solid dispersions (Leuner and Dressman, 2000). In some cases, a combination of these methods was used (Li et al., 1998). These methods, however, possess their own shortcomings.

Cyclodextrins, cyclic oligosaccharides derived from starch, display their high potential for improving solubility and bioavailability by forming an inclusion complex with a number of drugs (Loftsson and Brewster, 1996). The affinity between cyclodextrin and a given drug depends essentially on the physicochemical properties of both species. Unfortunately, different cyclodextrins exhibit diverse properties. The properties of naturally-occurring cyclodextrins depend mainly on their molecular feature. For chemically-modified cyclodextrins, their properties are not only based on the properties of the parent CDs but also the properties exerts by the substituted functional groups (Uekama et al., 1998).

Meloxicam is a long acting non-steroidal anti-inflammatory drug (NSAID). It was introduced and marketed for the treatment of osteoarthritis and musculoskeletal disorders. It is more interesting that meloxicam recently showed chemopreventive and chemosuppressive activities on various cancer cell line (Banerjee and Sarkar, 2002). This might lead to an important role of this drug for cancer treatment. Unfortunately, meloxicam possesses very low water solubility. Although the value of 270 microgram/ml in buffered solution of pH 7.0 was documented (Luger et al., 1996), the extremely lower values of 1.30 microgram/ml in solution of pH 1.2 and 7.0 microgram/ml in simulated intestinal fluid were also reported (Yazdanian et al., 2004). Furthermore, in vitro dissolution study revealed that only 38% of the drug was released from tablets (Nath and Kumar, 2000). The idea to improve the solubility of

meloxicam by cyclodextrin inclusion complexes is not new, this attempt has been made using β–cyclodextrin showing that the anti-inflammatory activity of the complex was better than the pure drug (Baboota and Agarwal, 2003). However, the inclusion complex approach using the other cyclodextrins has not been published. Meloxicam can exist in diverse structures depending on the pH value of the medium (Luger et al., 1996). The different prototropic forms show different affinity to cyclodextrins.

It is therefore, necessary to investigate the possibility of the complex formation between meloxicam and other types of cyclodextrins using β–cyclodextrin as a positive control. The investigation can be carried out basically by experiment. The experimental data obtained could provide specific information on meloxicam-cyclodextrin complexes. It can help making decisions for choosing the proper type of cyclodextrins for improving the meloxicam solubility. However, the experiment is usually time-consuming; the data obtained is rather specific to an investigated drug and could not anticipate the property of others. Moreover, it is quite difficult to carry out the experiment in the case, when the solubility of the drug is very low.

The Molecular Modeling, a method of theoretical prediction was also performed in order to get insight into the conformation of meloxicam-cyclodextrin inclusion complexes and to obtain theoretical models which could be applied to predict the stability of the complexes between cyclodextrins and other uninvestigated drugs.

1.2 Literature review

1.2.1 Cyclodextrin inclusion complexes

Mechanism of complex formation

The applications of cyclodextrins (CDs) in the broad area of pharmacy and pharmaceutical technology are due to their capability to form complexes (host–guest complexes) with a number of hydrophobic compounds. The phenomenon is known as molecular encapsulation or inclusion complex formation (Szejtli, 1998). The physicochemical properties of the guest change evidently upon entrapment in the CD cavity and that leads to a wide variety of inclusion complex applications (Hashimoto, 2002).

The complex formation between CD and a guest compound is known as non-covalent interaction. No covalent bond is formed or broken during the process. The main driving force of the complex formation is the release of enthalpy-rich water molecules from the CD cavity (Szejtli, 1998). Nevertheless, it is well-recognized that in addition to the release of high energetic water molecules, the complex formation is concurrently contributed by the other forces including the electrostatic interaction, van der Waals interaction, hydrophobic interaction, hydrogen bonding, charge-transfer interaction, release of conformational strain energy (Loftsson and Brewster, 1996). However, until now, it is still controversy for pointing out the most significance among these forces.

An extensive review article (Liu and Guo, 2002) demonstrated that van der Waals interaction and hydrophobic interaction are main driving forces for cyclodextrin complexation, excluding those contributed by the release of

conformational strain energy and the release of the cavity-bound high energy water which are energetically unfavorable. The contribution by electrostatic interaction and hydrogen bonding are justified for the significant effect on the conformation of a particular inclusion complex. Liu and his co-workers presented evidences to support that the charge-transfer interaction occurs as a driving force between benzoic acid, nitrobenzene and alpha-cyclodextrin (ACD) (Liu et al., 2001). In the case of β-carotene complexes with β-cyclodextrin (BCD) and γ-cyclodextrin (GCD), a large, micelle-like aggregate rather than the classical inclusion complex was suggested (Mele et al., 1998).

Preparation and characterization of inclusion complexes

Cyclodextrin-drug inclusion complexes can be prepared by several methods. The most useful method is characterized by the complete inclusion of the drug into the cyclodextrin cavity. When the objective of the complex formation is to enhance the dissolution rate or solubility of the drug, the method which is able to reduce the crystallinity of the drug or cyclodextrin during the process should be preferable. The popular methods for preparing the drug-CDs inclusion complexes are kneading method, co-precipitation method, evaporation method, spray-drying and freeze-drying method. Among these, freeze-drying successfully introduces the inclusion of a drug into CD cavity. Moreover, during the process of preparation, the drug and CDs are completely changed to amorphous state (Perdomo-Lopez et al, 2002; Reddy et al., 2004). Nevertheless, Vandelli and his colleagues suggested that for the formulation development, evaporation is superior to lyophilization method in the case of hydrodeoxycholic acid and hydroxypropyl-β-cyclodextrin (HPBCD) inclusion

complexes because of better dissolution improvement but, lower cost of preparation (Vandelli et al., 2000). Babu and Pandit prepared inclusion complexes of bupronolol in different CDs namely BCD, HPBCD and partially methylated-β-cyclodextrin (MeBCD) by evaporation method and characterized the complexes using X-ray powdered diffractometry (XPD) and differential scanning calorimetry (DSC). Both methods fully supported the formation of the inclusion complex (Babu and Pandit, 2004). A 1:1 molar ratio of piroxicam-HPBCD inclusion complex was prepared using freeze-drying method and characterized by DCS, XPD and Fourier transform infrared spectroscopy. The complex showed faster release from the buccoadhesive tablets than its physical mixture (Jug and Becirevie-Lacan, 2004). Veiga and Merino reported that the inclusion complexes of oxyphenbutazone and various CDs were successfully prepared by kneading method and XPD was shown as a very useful tool for characterizing these complexes (Veiga and Merino, 2002).

Besides the previously mentioned methods, the existence of inclusion complexes can be evidenced by the dissolution studies (Fernandes et al., 2002), nuclear magnetic resonance, ^{1}H-NMR and ^{13}C-NMR (Astilean et al., 1996; Mura et al., 1998; Van Santvliet et al., 1998). An application of NMR spectroscopy in cyclodextrin inclusion complex was extensively reviewed (Schneider et al., 1998).

Molecular modeling is one of the most useful tools used for characterizing the inclusion complex formation. It can not only evidence the existence of the complex but also provide information on the geometric feature of the complexes. MM2 calculations supported by NMR spectroscopy indicated that it was the thiadiazole group of sulfamethizole which is included into BCD and HPBCD cavity (Pose-Vilarnovo et al., 2001). The Density Functional Theory calculation combined with

Monte Carlo method was used to investigate the structural features of artemisinin-BCD inclusion complexes. The results were in agreement with those from Circular Dichroism spectroscopy (Marconi et al., 2004).

Stability constants of cyclodextrin inclusion complexes

Generally, the determination of the stability constant, K value can be accomplished by titrating the change in physicochemical properties of the guests as the function of CD concentration. Accordingly, a number of methods have been reported including: calorimetric titration (El Gezawi et.al., 1988; Braibanti et. al., 1998), spectroscopic methods such as electron absorption spectroscopy (Dalmora and Oliveira, 1999; Lui et. al., 2001), circular dichroism (Uekama et. al, 1998; Marconi et. al., 2004), fluorescence spectroscopy (Escandar, 1999; Tran et. al., 2002; Zhang et al., 2003), nuclear magnetic resonance (Shaomin et. al., 2003; Rozou et. al., 2004); potentiometric titration (Junquera et. al., 1999), gas- and liquid- chromatography (Ravelet et al., 2002; Rozou et. al., 2004); stability measurement (Masson et al., 1998; Walker et. al., 1998) and solubility determination using phase solubility diagram (Jug and Becirevic-Lacan, 2004; Saetern et. al., 2004). Among these methods, the calorimetry possesses advantages over the others, because the K values can reaction enthalpy change can be determined directly and simultaneously at any constant temperature.

Besides the different methods for the determination, the K value is also influenced by several factors which affect the complex formation, such as temperature, the pH of the medium and the presence of the other components.

Although the same CD derivatives is used, the K values differ when the degree of the substitution alters (Blanchard and Proniuk, 1999; Kopecky et. al., 2001).

The K value of piroxciam-BCD complex determined by fluorescence spectroscopy is 28000 M^{-1}, (Xiliang et al., 2003) whereas it is 3467 M^{-1} obtained from flow microcalorimetric method (Braibanti, 1998). Dalmora and Oliveira reported different K values of piroxicam-BCD complexes obtained from solubility study but at different pH conditions and these values (30-90 M^{-1}) were markedly lower than those obtained from the above mentioned methods. (Dalmora and Oliveira, 1999)

The ionization of a drug has significant effect on the complex formation. The unionized drug can bind stronger to CD than the ionized form due to the existence of hydrophobic interaction.

The hydrophobicity of the guest molecules also plays great role on the inclusion complex formation. The stability constants of the drugs and BCD complexes which in range of ibuprofen> naproxen>ketoprofen was attributed by decreasing series of hydrophobicity of the drugs (Mura et al., 1998).

The complex formation between a guest and a CD is a shape/size-matching phenomenon (Liu and Guo, 2004). Chemical modification of the parent CDs usually increases the complex ability, not only enlarging the cavity volume but the hydrophobicity of the cavity is increased. A number of studies illustrate better complex efficiency between the guests and modified CDs than the parent CDs (Dollo et al., 1996; Jarho et al., 1996; Hanna et al., 2003). The stability constants between flutamide and ACD and BCD inclusion complexes were lower than those obtained between the drug and the methylated derivatives, di- and trimethylated BCD (Taraszewska et al. 2003). Cyproterone formed more stable inclusion complexes with

BCD derivatives namely HPBCD, dimethylated BCD and randomly methylated BCD than the parent BCD (De Hassonville et al., 2002). Loukas and his co-workers demonstrated the lower K values between haloperidol and HPBCD than those shown by the drug and MeBCD. This finding was ascribed to less bulkiness of methyl substituent groups which could play less steric hindrance effect for the drug inclusion. (Loukas et al., 1997)

The effect of ionization of drugs on the stability constants of the complexes was reported (Okimoto et al., 1996). For seven ionizable drugs, the K values between the anionic drugs and CDs were comparable but, the values obtained between cationic drugs and anionic sulfobutylether-BCD were substantially higher than those of the drugs and neutral HPBCD.

Thermodynamic studies of the inclusion complexes

The complexation process is driven by enthalpy, exhibiting large and negative enthalpic changes and either positive or negative entropy changes (Li et al., 2003; Cerchiara et al., 2003). Theoretically, the negative enthalpic change results from van der Waals interaction and hydrogen bonding between guests and the cavities of cyclodextrins. In some cases the negative entropy change is attained due to the steric barrier caused by molecular geometrical shape and the limit of CD cavity to the freedom of shift and rotation of guest molecules. The positive entropy change is attributed by the enthalpy-entropy compensation phenomena (Sugihara and Hisatomi, 1999; Liu and Guo, 2004).

From the equilibrium constant, K the thermodynamic parameters of the inclusion reaction can be calculated. The change of the free reaction energy (ΔG) can be

directly calculated from the equilibrium constant and is temperature dependent. From the temperature dependency of the equilibrium constant the other thermodynamic parameters, the reaction enthalpy change (ΔH), and indirectly the reaction entropy change (ΔS), can be estimated. Both parameters are nearly temperature independent.

The effect of pH on thermodynamic parameters of ibuprofen has been demonstrated, the decreasing in enthalpy change at higher pH was attributed by weaker interaction of the ionized drug with CD compared to the unionized drug. The pH also affected the driving forces of the complex formation which was changed from entropically driven at pH 5.0 to enthalpically driven at higher pH. (Perlovich et al., 2003). The thermodynamic parameters of a series of drugs and other compounds have been summarized (Rekharsky and Inoue., 1998).

1.2.2 Application of cyclodextrin inclusion complexes

The CD inclusion complexes have been extensively applied in pharmaceutical area. This is reflected by a large number of CDs-related citations of nearly one-fourth of all applications (Szejtli, 1998).

Solubility and bioavailability enhancement

The inclusion complex formation of a drug with cyclodextrin alters the hydrophilic nature of the drug molecule by which the water solubility, the dissolution as well as the bioavailability of the drug are generally improved. The solubility and/or bioavailability improvement of the following drugs by inclusion complex formation with CDs has been demonstrated: phynyltoin (Savolainen et al., 1998); albendazole (Evrard et al., 2002); Cyproterone (De Hassonville et al., 2002); rufloxacin (Cappello

et al., 2002); sertaconazole (Perdomo-Lopez et al., 2002); carprofen (Chen et al., 2003); celecoxib (Sinha et al., 2005).

Stability improvement

The chemical reactivity of a drug after inclusion into the CD cavity is influenced in different ways. CDs can retard the degradation of an encapsulated drug by protecting the drug's labile group from environment or from the attraction by other reactants. In the cases, where the labile group of the drug is not fully concealed, the complex formation plays no effect of the drug decomposition. On the other hand, CD can accelerate the degradation rate of a drug by enzymatic-like catalysis. However, the change in chemical activity resulted by inclusion complex formation could provide advantages in CD applications. Firstly, the stability of the complex can be determined by titrating such changes. Secondly, which is more important is providing means for stabilizing labile compounds.

The photodegradation of the drugs was suppressed by forming inclusion complex with CDs e.g., DY-9760e (Nagase et al., 2001), nifedipine (Bayomi et al., 2002), or UVA and UVB filters (Fenyvesi et al., 2004). The stability of taxol from hydrolytic degradation was improved by forming inclusion complexes with HPBCD (Dordunoo and Burt, 1996). The degradation rate constant of dexamethasone acetate in the inclusion complexes with BCD and HPBCD were 3 and 10 times lower than the pure drug (Vianna et al., 1998). However, the contrary effect of CDs has been demonstrated. Although BCD, HPBCD and MeBCD increased the solubility of dihydroergotamine mesilate, they unfortunately exerted unfavorable effects on the drug stability. The degradation rate of the drug in BCD complex was similar to the

pure drug, but it was lower than the pure drug when complexed with HPBCD (Helm et al., 1995).

Carriers for drug delivery systems

The role of CDs as carriers for controlled drug release for special delivery systems such as for peptides and protein delivery and for site-specific drug delivery to brain, colon and cell surface has been extensively discussed (Uekama et al., 1998; Irie and Uekama, 1999). The usage of CDs combined with hydrophobic polymers can modify the release of drugs (Duchene et al., 1999; Fernandes and Veiga, 2002). The most interesting application of CDs in drug delivery systems is their ability to deliver agents for gene therapy such as plasmids, viral vectors and antisense contracts (Singh et al., 2002).

Analytical applications

CDs can be used in separation process due to their abilities to differentiate isomers, functional groups and enantiomers by inclusion complex formation (Kim and Park, 1998; Pak et al., 1998; Zhenming et al., 2003;). CDs are also used in high-performance liquid chromatography as stationary phases bonded to solid support or as mobile phase additives in capillary electrophoresis for the separation of chiral compounds. Recently, CDs have been used as fluorescence enhancer in HPLC for improving the detection limits of alflatoxins (Dallasta et al., 2003).

1.2.3 Applications of molecular modeling for cyclodextrin inclusion complexes

In order to get a better understanding of the inclusion complex formation, the theoretical modeling including the multivariate quantitative structure-activity relationship analyses, molecular mechanics (MM), molecular dynamics (MD) and more recently, quantum mechanics (QM) methods have been applied.

These methods are useful in predicting the binding constants of the inclusion complexation as well as illustrating which driving forces are important in particular complexation systems.

A correlation between the experimental molecular parameters of benzene derivatives and the association constants with alpha-CD and BCD was established (Guo et al., 1998). Linear and non-linear models for the prediction of the free energy of the complexes between various CDs and guest molecules were developed. These models give some insight into the complexation of parent CDs and a number of compounds. (Klein et al., 2000a; Klein et al., 2000b)

The applications of MM, MD and QM methods for studying cyclodextrins complexes were illustrated (Lipkowitz, 1998). Liu and his co-workers (Liu et al., 2001) applied PM3 calculations to study the driving forces of alpha-CD and benzoic acid and nitrobenzene complexes and presented the charge-charge transfer interactions as a driving force of the complexes. Peeters and his co-workers applied the molecular mechanics calculations to propose stoichiometric ratio of itraconazole-HPBCD inclusion complexes and HPBCD (Peeters et al., 2002). The MM2 force field method was used to verify the preferred orientation of sertaconazole and gamma-CD complex (Perdomo-Lopez et al., 2002).

1.3 Theories and principles

1.3.1 Introdution to Cyclodextrins

Cyclodextrins (CDs) were first discovered by Villers in 1891 by digesting starch using *Bacillus amylobacto*r. Thereafter, Schardinger identified these naturally occurring CDs and demonstrated them as alpha, beta and gamma cyclodextrins. These compounds were once known as Schardinger dextrins. During the period of 1911-1935, Pringsheim, a German scientist demonstrated the ability of these compounds to form complexes with a number of chemicals; he was thereafter recognized as the leader in the CDs research areas.

Natural cyclodextrins are now produced from starch by glycosyltransferase (CGTase) enzyme obtained from certain species of microorganisms. After being hydrolyzed, the amylase helix joined their ends together, generating a cyclic oligosaccharide or cycloamylose.

Structurally, CDs are cyclic oligosaccharides. The naturally-occuring CDs are composed of six, seven or eight D-glucose units joined by α-1, 4-glycosidic linkages. These CDs are known as alpha-CD (ACD), beta-CD (BCD) and gamma-CD (GCD). The most interesting molecular feature of CDs is the doughnut-like shape with a hydrophobic cavity and a hydrophilic exterior. The wider end of the CD molecule is presented by the secondary hydroxyl groups at C2 and C3, whereas the primary hydroxyl groups at C6 are located at the narrow end (Figure 1). The CD cavity is characterized by its highly hydrophobic nature, since the inside wall of the cavity is coated with hydrogen atoms from methylene groups. The polarity within the cavity has been estimated to be resembled to that of aqueous ethanolic solution.

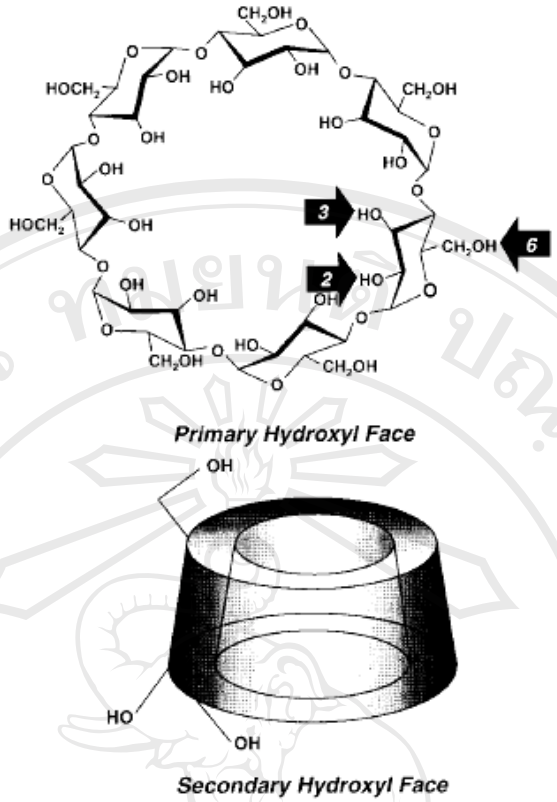


Figure 1 Structure of BCD showing the hydroxyl groups.

Source: Uekama et al., 1998.

Structures and Physicochemical properties of cyclodextrins

Table 1 Characteristics of cyclodextrins

	ACD	BCD	GCD
Number of glucose units	6	7	8
Molecular weight	972	1135	1297
Solubility in water; g/100ml	14.5	1.85	23.2
$[\alpha]_D$ 25°C	150±0.5	162.5±0.5	177.4±0.5
Cavity diameter, Angstrom	4.7-5.3	6.0-6.5	7.5-8.3
Height of torus, Angstrom	7.9±0.1	7.9±0.1	7.9±0.1
Outer diameter, Angstrom	14.6±0.4	15.4±0.4	17.5±0.4
Cavity volume, $(Angstrom)^3$	174	262	427
Crystal form (from water)	Hexagonal	Monoclinic	Prismatic
Crystal water, %weight	10.2	13.2-14.5	8.13-17.7
pK (by potentiometry) at 25°C	12.33	12.02	12.08

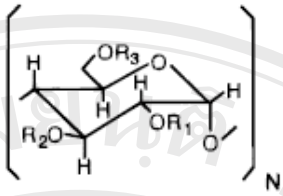
Source: Szejtli, 1998.

Cyclodextrin derivatives

In pharmaceutical applications, BCD is more commonly used than the other natural CDs (ACD and GCD) due to its appropriate cavity size for many drugs. However, the solubility enhancement application is restricted by its low aqueous solubility (1.85 g/100 ml). By the mid 1970s, chemically-modified CDs were synthesized and introduced in order to improve the aqueous solubility, to decrease the incidence of toxicity (Irie et al., 1997) as well as to improve their complexing ability (Suzuki et al., 1996). According to their substitutions at the rings, the modified CDs show diverse physico-chemical properties.

One of the most important applications of these CDs is to increase the solubility and the dissolution rate of low water-soluble drugs. Hydrophobic CD derivatives, such as 2,6-diethyl-β-CD slow down the dissolution rate of water-soluble drugs, therefore, they can be used as carriers for controlled release of drugs. Ionizable CD derivatives, such as O-carboxymethyl-BCD, O-carboxymethyl-O-ethyl-BCD, BCD-sulphate and sulfobutylether-β-CD have been reported to improve the inclusion ability, modifying the dissolution rate as well as alleviating the local irritation of drugs. Lists of chemically modified CDs according to the substituted groups are shown in Table 2.

Table 2 Classification of cyclodextrin derivatives



Cyclodextrin monomer

Cyclodextrin derivatives	R1	R2	R3
A. Hydrophilic derivatives			
Methylated cyclodextrins			
2,6-di-O- methylcyclodextrin	CH3	H	CH3
2,3,6-tri-O- methylcyclodextrin	CH3	CH3	CH3
randonly methylated cyclodextrin	R1, R2, R3 = H or CH3		
Hydroxyalkylated cyclodextrins			
2-hydroxyethylated cyclodextrin	R1, R2, R3 = H or CH2CH2OH		
2-hydroxypropyl cyclodextrin	R1,R2, R3 = H or CH2CH(OH)CH3		
2,3-dihydroxypropyl cyclodextrin	R1,R2,R3 = H or CH2CH(OH)CH3		
Branched cyclodextrins			
6-O-glucosylcyclodextrin	H	H	H or glucose
6-O-maltosylcyclodextrin	H	H	H or maltose
6-O-dimaltosylcyclodextrin	H	H	H or 2(maltose)
B. Hydrophobic derivatives			
Alkylated cyclodextrins			
2,6-di-ethylcyclodextrin	CH2	H	CH2
2,3,6-tri-ethylcyclodextrin	CH2	CH2	CH2
Acylated cyclodextrins			
2,3,6-tri-O-acetylcyclodextrin	COCH3	COCH3	COCH3
2,3,6-tri-O-propanoylcyclodextrin	COC2H5	COC2H5	COC2H5
2,3,6-tri-O-butanoylcyclodextrin	COC3H7	COC3H7	COC3H7
C. Ionizable derivatives			
6-O-(carboxymethyl)cyclodextrin	H	H	H or CH2COONa
Cyclodextrin sulfate	R1, R2, R3 = H or SO3Na		
Sulfobutylcyclodextrin	R1, R2,R3= H or (CH2)4SO3Na		

Source: Irie and Uekama, 1977.

Applications of cyclodextrins

Cyclodextrin is capable of entrapping a non-polar compound of optimal size and shape within its cavity resulting in the molecular encapsulation or inclusion complex formation. The inclusion complex exhibits physical, chemical, and biological properties that are dramatically changed from those of either the parent drug or cyclodextrin. The CD complex formation therefore has been extensively applied in wide area such as in pharmaceutical (Loftsson and Brewster, 1996; Chen et al., 2003), analytical and separation processes (Flood et al., 2000; Lu and Chen, 2002; Barr, et al., 2004), cosmetic (Fenyvesi et al., 2004), foods and flavors (Hedge, 1998; Dall'asta et al., 2003), agriculture, textiles (Szejtli, 2003) and environmental protection (Coly and Aaron, 1998; Singh et al., 2002). In pharmaceutical field, these complexes can be used to increase solubility and dissolution rate, decrease volatility, alter release rates, modify local irritation, and increase the stability of drugs. CDs have been demonstrated as a useful carrier for site specific drug delivery systems. Applications in peptides, protein formulations and gene therapy were also discussed (Uekama, 2002). Main benefits of CDs inclusion complexes in pharmaceutical field are briefly mentioned.

Solubility control

The water solubility of a poorly soluble guest molecule can be increased upon forming a complex with CD. When a guest molecule is included within the CD cavity, this molecule is entirely or partially surrounded by the CD molecule. The outer surface of the CD contributes the hydrophilic property for a guest. Thus, the solubility of the hydrophobic guest compound is subsequently increased. The solubilization ability of CDs can be quantitatively evaluated by the phase solubility method

developed by Higuchi and Connors (Higuchi and Connors, 1965). On the other hand, the drug-CD complex can slow down the dissolution rate of the included drug leads to the controlled-release formulation.

Bioavailability enhancement

As a result of the solubility improvement, the bioavailability of a poorly water soluble drug is improved by the inclusion complex formation. This is more pronounced for a drug where the absorption primarily depends on the dissolution step.

Stability improvement

The embedment of the whole or even a partial of the drug molecule into the CD cavity can help protecting the chemical labile group from the environment or the other chemical reactive species. The degradation of the drug could be evidently retarded.

Irritation Reduction

An active ingredient that irritate to mucous membrane such as to stomach, skin or eyes can be included within the CD cavity to alleviate its effect. The complex reduces the local irritating effect by avoiding the direct contact of the mucous membrane with such high free drug concentration. When the complex dissociate, the free drug is readily absorbed leaving the free drug concentration below the level that caused irritation.

Enhancement of Permeation

The cyclodextrin complexation leads to the increase in the permeation through biomembrane by increasing the drug concentration gradient over the membrane and this consequently increases in the flux through the membrane.

Improve the patient compliance

The unpleasant taste or odor of some substances can be masked by

encapsulation into the CD cavity.

Safety and toxicological considerations and regulatory status

The safety profiles of natural CDs have been extensively discussed. (Irie and Uekama, 1997). The toxicity studies have demonstrated that orally administered cyclodextrins are practically non-toxic because fewer amounts of CDs is absorbed from gastrointestinal tract. GCD and some CD derivatives, such as hydroxypropyl BCD, or sulfobutylether BCD have been shown to be safe for parenteral administration. Some toxicological studies revealed that the parent ACD, BCD and the methylated BCD are not recommended for parenteral formulations due to nephrotoxicity. However, for BCD, its monograph has been included in the European Pharmacopoeia, the United State Pharmacopeias/National Formulary and Japanese Pharmacopeias. It is considered as an essentially non-toxic and non-irritant excipient, authorized for food applications. A number of products containing BCD including non-parenteral pharmaceutical and food products have been approved for human used in Europe and in Japan. The European Scientific Committee for Foods (SCF, December 1996) evaluated BCD and assigned it an acceptable daily intake (ADI) of 5 mg/kg body weight.

Table 3 Toxicological data of some cyclodextrins

CDs	LD_{50}, oral in rat	LD_{50}, i.v. in rat
BCD	>5000 mg/kg	450-790 mg/kg
GCD	>> 8000 mg/kg	4000 mg/kg
HPBCD	>5000 mg/kg	>5000 mg/kg
MeBCD	>> 8000 mg/kg	1500-2100 mg/kg

1.3.2 Inclusion complex formation

A. Mechanism of complex formation

The main driving force of the complex formation is the release of the water molecules from the CD cavity. These water molecules are then displaced by the hydrophobic guest molecule. The hydrophobic interaction takes account to some extent. Upon the apolar association the CD constrain energy decrease to the lower energy state leading to the stable complex. No covalent bonds are involved in the complex formation process (Figure 2).

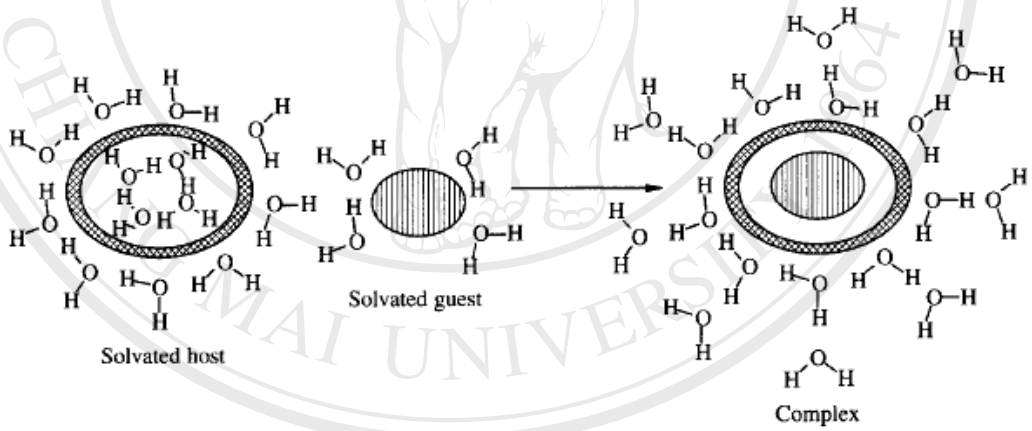


Figure 2 Mechanism of cyclodextrin complex formation
Source: Szejtli, 1998.

Steps of the inclusion complex formation:

- The approach of the guest to the CD molecule
- Break down of the water structure inside the CD ring and removal of some molecules from the ring.

- Break down of the water structure around that part of the guest molecule.
- Interaction of the substituents of the guest molecule with groups on the rim or the inside of the CDs.
- Possible formation of hydrogen bonds between guest and CD.

B. Requirements for complex formation

1. Geometric compatibility

The minimum requirement for the inclusion complex formation is that the guest molecule must fit entirely or at least partially into the CDs cavity. Stable complexes will not be formed with guest molecules which are too small to be enclosed by the CDs molecules because they will slip out of the cavity.

Complex formation is also impossible with guest molecules which are too bulky to enter into the CD cavity. However, if certain groups or side chains of the bulky molecules can penetrate into the cavity, complex formation remains possible.

Normally 1:1 (Drug: CD) complexes are formed, but when the guest is too long to find complete accommodation in one cavity and its other end is also amendable to complex formation, also 1:2 or higher complexes can be formed. It may also be possible to form 2:1 or three component complexes.

Most drug molecules tend to be entrapped better into the BCD than ACD, because the cavity diameter of BCD approximately 6 Angstrom can accommodate the aromatic groups of many drug molecules. In contrast, the cavity diameter of ACD is too small for a favorable fit. Interaction can also be seen between many drugs and ACD.

2. Polarity and charge

Both stereochemistry and polarity of the guest molecules determine whether inclusion may occur or not. In general, hydrophobic molecules or residuals rather than hydrophilic ones have higher affinity to the CDs cavity in aqueous solution.

3. Binding forces in the complexes

Despite force of the complex formation by hydrophobic interaction, the nature of the binding force is controversial. The interaction force of the inclusion complex formation can not be a classical non-polar binding. Usually, non-polar binding is characterized by a very favorable entropy change. Meanwhile, the inclusion complex formation is associated with a favorable enthalpy change and an unfavorable entropy change.

The CD complexes formed are stabilized by various intermolecular forces:

1. Van der Waals interaction between the guest and the host

   The Van der Waals forces here include both:

   - Permanent induced dipole-dipole interaction
   - London dispersion forces

2. Hydrogen bonding between guest and host
3. Release of water molecules in the complex formation

In aqueous solution, several water molecules are accommodated in the CD cavity. These water molecules can not form hydrogen bonds with one another as in the bulk of the solvent. Therefore, they are regarded as molecules of enhanced energy or enthalpy. Inclusion complex formation involves the replacement of these high enthalpy water molecules by guest compounds, resulting in a favorable enthalpy change (large negative ΔH)

4. Release of the strain energy in the macromolecular ring of the CD.

Change from the high energy conformation of CD-water complexes to the low energy conformation of CD-guest complexes

C. Methods of preparation of solid inclusion complexes

The guest-CD inclusion complex can be formed easily even if both are in solid state. However, some traces of water are still needed. Water is very important for the process. In addition to involving as the main driving force of the complex formation, it acts as solvent that brings both guest and CD molecules into solution, in which the complex formation more readily occurs. For very slightly soluble guest, sometimes, small amount of organic solvent are used in order to facilitate the solubility of the guest molecule upon complex formation process. There are several different methods of preparing inclusion complexes

The most common procedure is to stir or shake an aqueous solution of CDs with the solid guests or its solution. The solid complex is harvested by suitable drying process such as spray-drying, freeze-drying or evaporation under reduced pressure.

If the system has a B_s-type phase solubility diagram, the solid inclusion complex can be obtained by precipitation of the microcrystalline powder and this powder is subsequently separated by filtration.

Other methods to prepare solid inclusion complexes are:

Freeze-drying

Spray drying

Kneading

Co-precipitation

Neutralization

Miscellaneous methods such as grinding, melting, sealed heating method

D. Characterization of solid complexes

1. Detection of inclusion complexes in solid state

Detection of inclusion complexes in solid state can be performed using the following techniques

1. Powder X-ray diffraction

This method is useful in the case of liquid guest molecules since, liquids have no diffraction peaks of their own. When the pattern of a newly formed substance clearly differs from that of uncomplexed CD, complex formation is indicated.

In cases of solid guest compounds, a comparison has to be made between the diffractograms of the assumed complex and that of the simple blend mixture of the guest and the CD. Comparison of the diffractograms is only possible if the CD as well as guest are, before mixing, both treated under identical conditions as the assumed complex, because CD inclusion complex preparation process such as freeze-drying, grinding may alter the crystalline of the pure substances. This may lead to different diffraction patterns.

The diffraction pattern of the physical mixture is often the sum of those of each component whereas, the diffraction of the CD complexes are different from each constituent and lead to a new solid phase with a different diffractogram.

2. Single crystal X-ray structure analysis

This method may be used to determine the detailed inclusion structure or mode of interaction.

- Interaction between the guest and host molecules can be identified.

- The precise geometrical relationship can be established.

Although, single crystal X-ray analysis provides much information about inclusion complexes, this technique is too complicated for routine use.

3. Thermo-analytic methods or Thermal analysis including differential thermal analysis (DTA) and differential scanning calorimetry (DSC).

These methods determine whether the guest substance undergoes some change before the thermal degradation of CD. These changes may be: melting, evaporation, decomposition, oxidation or polymorphic transition. The existence of the inclusion complex can be detected by the disappearance of the characteristic peaks of the guest or in some cases, the broadening of the peaks.

4. Infrared (IR) spectroscopy

This technique is used to assess the interaction between CD and guest molecules in solid state. This method is not generally suitable to detect inclusion complexes and is less evident than other methods, because the CD bands are often changed only slightly upon complex formation, and if the fraction of guest molecules encapsulated in the complex is less than 25 %, bands which could be assigned to the included part of the guest molecules are easily masked by the bands of the spectrum of CD. The application of IR spectroscopy is limited to guests having some characteristic bands such as carbonyl- or sulphonyl- groups.

5. Scanning electron microscopy (SEM)

SEM is used to study the microscopic aspects of the raw materials (CD

and guest substances, respectively) and the products obtained (by co-precipitation or co-evaporation). Even if there is a clear difference between the crystallization state of the raw material and the product obtained by co-precipitation, this method is inadequate to affirm inclusion formation, but nevertheless helps to assess the existence of a single component in the preparations obtained.

6. Wettability and dissolution tests

The complexation between CD and lipophilic drugs often improves the wettability in water, but also addition of BCD to non-wettable solid enhances its wettability due to surface active like property of CD.

The measurement methods of the wettability of solid CD formulation includes:

1. The measurement of contact angle.

2. The powder sedimentation studies: by layering equal amount of the samples onto the surface of water, then photographing their sedimentation.

3. Registration of the upward migration of a colored front of the three open tubes containing the guest compound, a mixture of guest compound and CD and the inclusion complex.

4. Dissolution rate test:

When the assumed complex is dispersed in water, very rapid dissolution rate are based on this observation. The most often used dissolution rate tests are:

7.1 The rotating disk method

7.2 The disperse amount technique

The rotating disk method: The solid CD formulations are pressed into tablets with exact identical surfaces for the samples and these tablets are placed on a rotating disk apparatus in an aqueous medium. At appropriate time intervals, samples are withdrawn and analyzed for the guest content.

The disperse amount technique; powder is used instead of tablets.

2. Detection of inclusion complexes in solutions

2.1 Spectroscopic methods

2.1.1 Electron absorption spectroscopy

UV/Visible spectroscopy is a useful method to determine inclusion complexation when the process causes a change in the electron absorption spectrum of the guest molecules. In general, the spectral changes observed are similar to the effects caused by changes in polarity of a solvent, suggesting that the chromophore of the guest molecules is transferred from an aqueous medium to the non-polar CD cavity. These changes must be due to a perturbation of the electronic energy levels of the guest caused either by

- Direct interaction with the CD.
- Exclusion of solvating water molecules.
- Combination of these two effects.

Although frequently only small shift are observed in the UV spectra of inclusion complexes, this method is often used to detect the inclusion complexation.

2.1.2 Fluorescence spectroscopy

A difference in the fluorescent spectra of the fluorescent molecules in the absence or in the presence of CD in aqueous solutions may be obtained.

2.1.3 Circular dichroism spectroscopy

Circular dichroism is a useful method to detect CDs inclusion complexation in aqueous solution. When an achiral guest molecule is included into the asymmetric cavity of CD which consists of chiral glucose units, circular dichroism can be induced in the absorption bands of the optically inactive guest. Not only achiral guest molecules, but also chiral guest molecules may show changes in circular dichroism spectra upon the formation of inclusion complexes.

2.1.4 Nuclear Magnetic Resonance (MNR) spectroscopy

The most direct evidence for the inclusion of a guest into a CD cavity in solution is obtained by ^{1}H-NMR spectroscopy. ^{1}H-NMR may also used be to determine the direction of penetration of guest molecules into the CD cavity.

The H-3 and H-5 atoms of CDs, which directed towards the interior of the CD cavity, will show a significant upfield shift if the inclusion occurs. The H-1, H-2 and H-4 atoms, located on the exterior of the cavity will show only marginal upfield shifts.

Alternatively, if association takes place at the exterior of the torus, H-1, H-2 and H-4 shall be strongly shielded. The spectrum of the guest molecule may also be changed upon inclusion complex formation.

A similar method to investigate inclusion complex formation is ^{13}C-NMR spectroscopy. It is used to gain insight into the inclusion modes of inclusion complexes in aqueous solution.

The CDs induced change in the ^{13}C-chemical shift results from the electronically environmental effects of the CDs cavity.

2.1.5 Near infrared spectroscopy

NIR spectroscopy has been used extensively in recent years for chemical analysis and characterization of the inclusion complexes. Its popularity derives from the advantages of the technique such as wide applicability, noninvasive, nondestructive, real-time and on-line capabilities. NIR region covers the overtone and combination transitions of the C–H, O–H and N–H groups, and since all organic and most inorganic compounds possess at least one or more of these groups, the technique can, in principle, be used for the analysis of all organic and any inorganic compounds. Therefore, it can be used to investigate the CDs and guest interaction when the guest shows strong absorption bands in the NIR.

2.2 pH-potentiometric titration

If the guest molecule has a prototropic function, the potentiometric titration can be used to detect inclusion complex formation. Due to the fact that CDs usually favor the unionized guest molecules having a higher hydrophobicity rather than the ionized ones, the pKa value of an acidic guest molecule is usually increased, while that of basic ones is usually decreased by the binding to CDs.

2.3 Electrochemistry

2.3.1 Polarography

It is a suitable method to study inclusion complexation if the electron distribution of a complexed electroactive guest molecule in aqueous solution is different from that in the non-complexed state in aqueous solution and this change is polarograghically detected.

2.4 Microcalorimetry

Changes in thermodynamic properties due to inclusion complexation can be measured by microcalorimetry. The changes in enthalpy, entropy are associated with the change in behaviors of water following complex formation, and includes:

- A break down of the water structure within the cavity.
- Removal of the water molecule from the cavity.
- Restructuring of water around the guest molecule.
- Release of water into the bulk.

Other contributions to the overall energies of reaction are due to the restriction in rotation around the glycosidic linkage of the CDs when the guest molecule enters the cavity. Positive entropy suggests significant contribution from the redistribution of water. Large enthalpy should be consistent with dipole interaction and H-bond formation.

2.5 Solubility Methods

The solubility method using phase-solubility diagram is widely used to characterize the guest-CD complex. The phase solubility method was introduced by Higuchi and Connors (Higuchi and Connors, 1965). It is a plot of solubility of guest, [D] as a function of CD concentrations, [CD] (Figure 3).

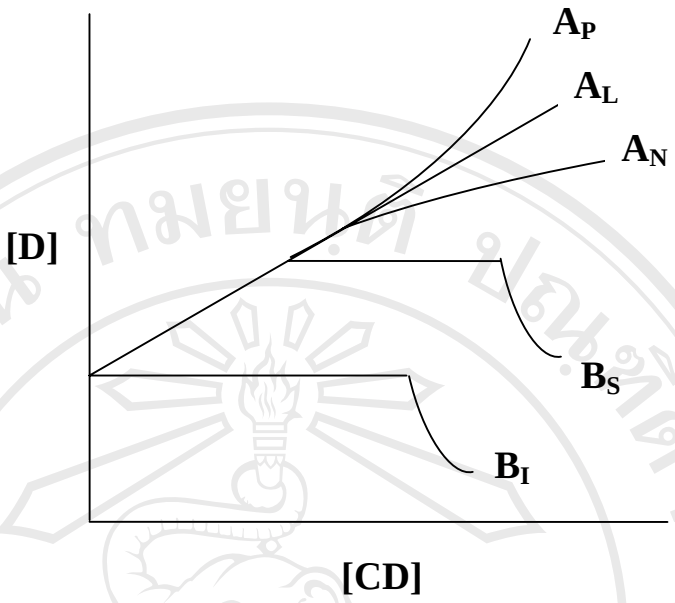


Figure 3 Typical phase solubility diagrams

According to Higuchi and Connors the phase solubility diagram can be classified into 2 types depending on the complex solubility. Type A phase solubility diagram is obtained when a soluble complex is formed. Type B is obtained when a complex with limited solubility is formed. Type A can be further divided into subtypes A_L, A_P, and A_N. The A_L type is assigned when the complex solubility increases linearly with CD concentration. In the case of A_P and A_N types, the solubility profiles deviate positively or negatively, respectively, from the straight line. Generally, the complex of 1:1 stoichiometric molar ratio shows the A_L type diagram. The A_P type signifies the higher order complex formation in which more than one CD molecules are involved in the complexation. Systems of A_N type are rather complicated due to a significant solute-solvent interaction existing during complex formation.

The B_S type solubility curve is characterized by the initial increasing of the complex, followed by a plateau region and then a decrease in the solubility at higher CD concentrations. At plateau region, the solubility of the complex is independent on the CD concentration implying that the saturated solubility point of the complex is reached. The decline phase is resulted from a precipitation of the complex and/or CD. The B_I type diagram is indicative of the formation of insoluble complexes in water.

The stability constant and stoichiometry of complexes are determined by analyzing quantitatively the phase solubility diagram.

E. Stability constant of inclusion complex

The stability constant or the binding constant of the complex, K is a useful parameter for indicating the stability of the complex, signifying the affinity between a guest and a CD and also notifying the complexation ability of various CDs for a given guest.

The K value can be quantitatively determined from experiments. The determination is accomplished by monitoring the changes in the physicochemical properties (solubility, spectral properties etc.) of the guest as a function of CD concentrations. It can be obtained from several methods such as solubility method, fluorometry, potentiometric titration, conductivity or stability method for example and depends on what property has been significantly altered upon the complex formation.

The magnitude of the stability constant for most drug substances and CDs range from 0 to 100,000 M^{-1} (Rajewski and Stella, 1996). However, the high $K_{1:1}$ value of 744,877 M^{-1} between the anandamide and DiMeBCD was reported (Jarho et al., 1996). The K values of 200-5000 M^{-1} are considered to be adequate for the

complexes which could contribute to the improvement of the bioavailability of poorly water soluble drugs.

A statistical analysis on a large number of drug: CD complexes revealed that the mean K value was 123, 490, and 525 M^{-1} for ACD, BCD and GCD respectively (Burnett and Connors, 2000).

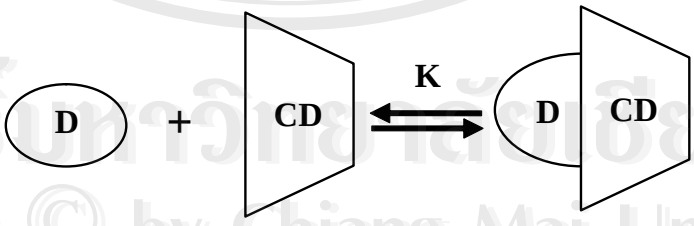
The K value of a new compound can be predicted by molecular modeling. This approach has become more interesting tool to explore the guest-host interaction. The determination of K value by experiment is sometimes restricted when the solubility of the guest is very low or less stable.

From phase solubility diagram, the K value of the complex of 1:1 stoichimetric ratio can be calculated by the following relationship

$$K_{1:1} = \text{Slope}/So*(1\text{-slope})$$

Where So is the intrinsic solubility of the guest in the absence of CD.

The complexation ability is obtained from:



$$D + CD \underset{}{\overset{K}{\rightleftharpoons}} \frac{[D-CD]}{[CD]} \frac{1}{[D]}$$

$$K = \frac{[D - CD]}{[D][CD]}$$

[D] is intrinsic solubility of the drug, So

Complexation efficacy = K. So = [D-CD]/[CD]

Figure 4 Complexation efficiency

F. Dissociation of inclusion complexes

Besides, the formation of the complex, the dissociation which leads to the guest-release is also an important factor to consider on the utilization of CD complexes. The complex should be stable enough for harvesting meanwhile; it should dissociate to give a desirable amount of free drug when needed.

There are several factors affecting the complex dissociation either increasing or decreasing the speed of guest-release. The increasing factors are listed as followed however; the opposite effect will decrease the release rate.

• Dilution effect

This is very effective for dissociating the weak CD-guest complexes and even has considerable effect on strong complexes when diluting with non-polar solvent.

• Guest binding to plasma protein and tissue components when the complexes are administered

Some guest molecules have greater affinity to the plasma protein than to CD, leading to release of the drug from CD cavity

• CD elimination of CD *in vivo*

When the CD is destroyed, the guest will be released to the medium.

• Temperature and pH

The dissociation of the complex is greatly affected by temperature and pH change. The effect of a given temperature or pH differs greatly from complex to complex.

• Competitive displacement

This occurs if molecules in the solvent or the third component have a greater affinity for binding in the CD-cavity than the guest molecule. This effect is especially pronounced even in strong complexes.

• The stability of the complex

The guest release rate is faster if the CD-guest complex is weak.

• Ionization of the guest molecule

A faster release also occurs if the guest molecule is ionized, because ionization increases hydrophilicity.

1.3.3 Introduction to piroxicam

Piroxicam (4-hydroxy-2-methyl-*N*-2-pyridyl-2*H*-1,2-benzothiazine-3-carboxamide-1,1-dioxide) (Figure 5), is a non-steroidal anti-inflammatory drug (NSAID), known as cyclooxygenase-1 selective inhibitor. It is recommended for the treatment of rheumatic disorders and other chronic arthritic symptoms.

Figure 5 Structure of piroxicam

Physicochemical properties

Piroxicam is an amphoteric drug containing two ionizable groups; the acidic enolic group and the 2-pyridyl group. Accordingly, it exhibits two distinct pKa values responsible for the ionization of each group at 1.86 and 5.46 respectively (Bernhard and Zimmermann, 1984). The reported pKa values of piroxicam were quite diverse depending on the type of solvent used. The pKa1 and pKa2 in dioxane/water and in ethanol/water were reported as 1.86 and 6.30; 1.90 and 5.26 respectively. (Wiseman et al., 1976; Tsai et al., 1993). The water solubility is relatively pH-dependent and is substantially increased at lower pH and at higher pH value. The molecular size of piroxicam is approximately 6.0 Angstrom wide and 13.7 Angstrom long (Escandar, 1999). Its melting point of the stable cubic crystalline form is 201°C whereas it is 197 °C for the metastable needle-form.

1.3.4 Introdution to Meloixcam

Meloxicam [4-hydroxy-2-methyl-*N*-(5-methyl-2-thiazolyl)-*2H*-1,2-benzothiazine-3-carboxamide-1,1-dioxide] is a novel nonsteroidal anti-inflammatory drug (NSAID) that inhibits prostaglandin synthesis via relatively selective inhibition of cyclo-oxygenase-2 (COX-2). It is generally considered to be as preferential but not selective COX-2 inhibitor.

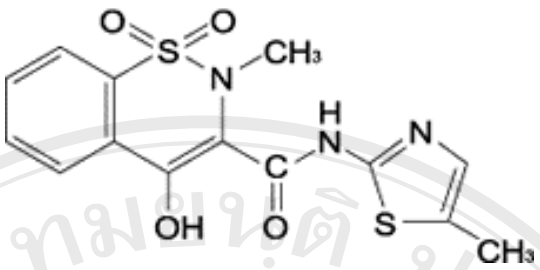


Figure 6 Structure of meloxicam

Meloxicam is structural related to piroxicam; the pyridin-2-yl ring of piroxicam is replaced by a 5-methylthiazol-2-yl ring. (Figure 6). Although their structures are closely related, the selectivity for COX-2 is very different. The 5-methylthiazol-2-yl ring is responsible for the selectivity for COX-2. It was demonstrated that a slight difference in chemical modification in this ring results in the difference in COX-2 selectivity. Meloxicam showed higher preferential inhibition of COX-2 relative to COX-1 compared to its 4′-epimer (Pairat et al., 1998)

Physicochemical properties

Similar to piroxicam, meloxicam is an amphoteric drug showing two pKa values at 1.09 and 4.18 from UV spectrophotometric determination. The different values of 1.29 and 4.35 determined by solubility-pH profiles were also reported. Its molecular conformation and water solubility is drastically affected by pH change. At pH greater than 4.18, meloxicam can exist in the enol or zwitterionic form, depending on the polarity of the solvent. In aqueous solution the zwitterionic form is the predominant species. At low pH, the cationic form exists. The anion is predominant at pH 7.4 or higher.

1.3.5 Molecular modeling (Lipkowitz, 1998; Selassie, 2003)

Molecular Modeling is a new discipline, which combines the methods of theoretical chemistry with the facilities of modern computer technologies. It consists of a series of methods, including the calculations of molecular geometries and molecular properties on various levels of accuracy, the presentation of the geometries together with the corresponding properties and the establishing of convenient correlations between biological effects and molecular properties, so called Quantitative Structure Activity Relationships (QSAR) or Quantitative Structure Property Relationships (QSPR). Models obtained from such analyses allow the prediction of biological activities for new compounds and they are widely used in pharmaceutical research and drug design.

The molecular information such as geometries, energies and structure-associated properties can be calculated and displayed using available computer programs. The relationship between these calculated properties and a measured biological activity or chemical activity is established using an appropriate statistical principles.

General form of a QSAR model:

$$\text{Activity} = \text{Constant} + (c_1 * P_1) + (c_2 * P_2) + (c_3 * P_3) + \ldots\ldots (c_i * P_j)$$

Where c_i are the least square multiple regression coefficients and P_j are the molecular descriptors.

In practice, a model is derived from a set of compounds which is the so called training set. The compounds associated with their activity values are collected either by performing experiments or by literature search. Theoretically, the molecular structure of these compounds should be diverse in order to provide a model of wide

prediction range. The quality of the model is essentially depending on the quality of the data used to develop the model. For the data obtained from own-designed experiments, the repeated values for any observation should be of high precision with relatively small errors. In cases where the data were collected from different experimental sources, the relevant criteria of acceptance should be set previously. Only data in agreement with the criteria should be included in the training set. Furthermore, the data analysis should be justified by appropriate statistical principles including confidence intervals of all regression coefficients and leave-one-out (LOO) cross-validation to assure the high quality of the obtained model.

From the model the activity of a new compound can be anticipated by computing its molecular descriptors and substituting these values into the model. The recommended molecular descriptors included in the model are steric parameters which provide information about molecular size and shape, electronic parameters and hydrophobicity parameters.

Some methods for the calculation of molecular conformations and molecular properties used for this method are briefly discussed.

Quantum chemical method

These methods consist of semiempirical methods and ab initio methods. They are used for energy minimization or geometry minimization, thus providing the information about structural and electronic features of the molecule. The basic principle of these methods is the Schrödinger equation, where a wave function is introduced to describe the properties of an electron in the field of a nucleus. This equation can only be solved exactly for the hydrogen atom. For all other atoms and

molecules various approximations are applied, partly compensated by empirical parameters obtained from experiments. The most accurate methods are ab initio and density functional models, which do not use experimental parameters. But such calculations are very time consuming and need large computer resources.

Molecular mechanics method

This method is known as a non-quantum mechanic method used for determining structures, energies and molecular properties which do not depend on electronic prpperties. The calculation performed based on empirical functions and parameters of the potential energy depending on internal coordinates such as bond length, bond angles and torsion angles. The potential energy is minimized by moving the particles towards their equilibrium position.

Molecular dynamics methods

The theory implemented is based on the equation of motion by Newtonian law. The kinetic energy by increasing temperature is applied to generate the molecular motion over the molecular potential energy map. Thus provides many trial conformational states along the way of motion.

Monte Carlo Simulation methods

For a given collection of particles, its energy of configuration is initially computed then compared to the energy of a new configuration which is obtained

randomly by moving and move one or more particles. A large number of random motions are made until the lowest-energy configuration is obtained.

1.4 Objectives of the study

1. To determine thermodynamic parameters of drug-cyclodextrin inclusion complex by solubility study
2. To investigate the factors affecting the inclusion complex formation between drug and cyclodextrins.
3. To characterize the drug-cyclodextrin the inclusion complexes.
4. To apply molecular modeling on drug-cyclodextrin inclusion complex formation.

1.5 Scope of the study

1. Model drug : piroxicam and meloxicam
2. Cyclodextrins :

   Natural cyclodextrins : BCD, GCD

   Chemical modified cyclodextrins : HPBCD, HPGCD, MeBCD
3. The factors affecting the inclusion complexes

   3.1 In solution : temperature, pH, cyclodextrin type

   3.2 In solid state : cyclodextrin type, method of preparation, drug to cyclodextrin molar ratio
4. Characterization methods :

   4.1 Dissolution study

   4.2 Differential scanning calorimetry (DSC)

4.3 X-ray powdered diffractometry (XPD)

4.4 Fourier transform infrared spectroscopy (FTIR)

4.5 Near infrared spectroscopy (NIR)

5. Molecular modeling

5.1 Development of quantitative structure property relationships (QSPR) for prediction the stability constants of the drug and CD complexes.

5.2 Application of molecular calculations for geometry optimization of drug-cyclodextins complexes